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# EFFECTS OF SALINITY AND STARVATION ON THE UPTAKE AND UTILIZATION OF DISSOLVED GLYCINE BY *AURELIA AURITA* POLYPS

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The active uptake of amino acids from dilute solution by marine invertebrates of a number of phyla is a well documented phenomenon that has been adequately reviewed elsewhere (*e.g.,* Anderson and Stephens, 1969; Johannes, Coward and Webb, 1969; Southward and Southward, 1970; Stephens, 1972). Webb, Schimpf and Olmon (1972) have characterized the free amino acid (FAA) pools of recently fed *Aurelia aurita, Chrysaora quinquecirrha* and *Cyanea capillata fulva* scyphistomae, and Raum (1970) has made similar observations on *Aurelia* polyps that had been starved for one month. Although the uptake of dissolved amino acids by cnidarians has been documented among the Anthozoa (Stephens, 1962) and the Hydrozoa (Stephens and Schinske, 1961), such reports are lacking for the Scyphozoa.

A major controversy has concerned the possible role of dissolved amino acids as an energy source for marine invertebrates, with (in recent years) Stephens (1967, 1968), Little and Gupta (1968), Southward and Southward (1968, 1970, 1972) and others maintaining that this is the case. Other workers have observed a net loss of FAA by marine invertebrates; Johannes *et al.* (1969) review and discuss much of this literature, stating that the net loss of these substances precludes their utilization as a significant source of nutrition.

Johannes *et al.* (1969), using $C^{14}$-labeled amino acids and ion exchange chromatography, monitored the total flux of FAA in the turbellarian *Bdelloura candida;* the latter technique was also used in determining the rates of release of FAA by starved turbellarians at different salinities (Webb, Johannes and Coward 1971). Stephens (1962, 1963, 1964) and Stephens and Virkar (1966) have examined the role of dissolved labeled amino acids in the energy budgets of a variety of marine invertebrates, emphasizing salinity induced effects. In the investigation of the significance of dissolved FAA as a supplemental energy source, an important extension of the above experiments is to examine the effects of starvation on the uptake, subsequent distribution and utilization of labeled amino acids over a range of salinity. Since glycine is the major constituent of the FAA pools of *Aurelia* polyps (Raum, 1970; Webb *et al.,* 1972), such experiments were undertaken using ecologically meaningful concentrations of glycine in seawater.

## Materials and Methods

Polyps of *Aurelia aurita* were obtained from the Virginia Institute of Marine Science, Gloucester Point; the animals were from a culture isolated by Spangenberg (1964) at Corpus Christi, Texas. Groups of scyphistomae were acclimated

to salinities of 10, 20, 30 and 40‰ (artificial seawater prepared according to the formula of Spangenberg, 1965) at room temperature (22°–24° C) for two months prior to the experiments. Iodide was deliberately withheld, lest effects of iodine induced metamorphosis (Spangenberg, 1967) complicate the experimental results. Polyps were fed *Artemia salina* nauplii twice weekly, and the water in the culture dishes was changed approximately six hours after feeding.

Preliminary experiments in which polyps were preincubated for 21 hours in streptomycin sulfate (200 mg/l seawater) indicated that bacterial contamination altered neither total glycine uptake nor the distribution of radioactivity in the various fractions. Therefore, to minimize external variables, streptomycin was not used in the main body of experiments, and the following decontamination procedures were utilized. Polyps were removed from the culture dishes either 48 or 288 hours after feeding, adhering debris was removed with a pipet, and the animals were washed four times with Millipore filtered (0.45 $\mu$ pore size) artificial seawater of the appropriate salinity.

Groups of 10 polyps of uniform size were transferred to sterile test tubes in 0.5 ml of sterile seawater of the acclimation salinity. To each tube was added 1.0 ml of sterile seawater of the acclimation salinity with a $C^{14}$ glycine (U.L.) concentration of 1.27 $\mu$ moles/l (0.1 $\mu$Ci/ml), giving a final glycine concentration of approximately 0.85 $\mu$ moles/l in the exposure medium. This concentration was felt to be ecologically realistic (*cf.* Stephens, 1963; Webb and Wood, 1967).

Scyphistomae were exposed to the labeled medium in sealed test tubes for one hour, quickly washed in 4 changes of sterile, unlabeled seawater, and incubated for an additional 23 hours in sterile, unlabeled seawater at room temperature. $CO_2$ was collected throughout the exposure and incubation periods in 10% KOH on ground glass rods imbedded in the stoppers sealing the individual test tubes. The KOH was rinsed into individual counting vials at the end of the incubation period and counted in Aquasol Universal L.C.S. Scintillator on a Beckman LS-200B liquid scintillation system. The polyps were again washed in unlabeled seawater and finally placed in 1.5 ml of 80% ethanol for 24 hours. Aliquots of the EtOH extracts (FAA) were then added to scintillation cocktail (6 g PPO/l toluene, added 2:1 to Triton X-100). The polyps were rinsed four times in clean ethanol, air dried briefly, placed in counting vials with 0.5 ml NCS Solubilizer and digested for 24 hours. Scintillation cocktail was then added and the pH adjusted to approximately 6 with glacial acetic acid. All samples were allowed to stand for 24 hours before counting, and were corrected for background and for quenching.

Experimental procedure prevented weighing the experimental animals, although mean dry weights for recently fed (48 h) and starved (288 h) polyps were determined using two replicates of three uniformly sized individuals from every experimental group. Polyps were removed from the culture dishes, rinsed briefly in glass distilled water, placed in groups of three on tared foil strips, dried for 24 hours at 55° C and weighed to the nearest 0.01 mg.

## Results

### *General*

Polyps increased in size and reproduced asexually at all experimental salinities, with the highest growth and reproductive rates occurring at 30‰, and the lowest

TABLE I

*$C^{14}$ glycine distribution and utilization (cpm/μg dry wt. ± S.D) by groups of 10* Aurelia *polyps at various salinities and times since feeding. Mean dry weight (μg/polyp) for each experimental group is given in parentheses*

| Fraction | 10‰ | | 20‰ | | 30‰ | | 40‰ | |
|---|---|---|---|---|---|---|---|---|
| | 48 h (29) | 288 h (23) | 48 h (42) | 288 h (39) | 48 h (43) | 288 h (27) | 48 h (33) | 288 h (30) |
| | n = 3 | n = 4 | n = 3 | n = 4 | n = 3 | n = 4 | n = 4 | n = 4 |
| $CO_2$ | 15.9 ± 1.5 | 45.7 ± 8.9 | 8.3 ± 1.1 | 19.7 ± 7.4 | 5.2 ± 0.7 | 15.4 ± 3.6 | 13.8 ± 2.0 | 16.5 ± 1.5 |
| FAA | 10.2 ± 2.3 | 10.0 ± 3.6 | 61.5 ± 10.3 | 67.0 ± 5.6 | 105.1 ± 14.8 | 77.6 ± 22.8 | 65.5 ± 24.3 | 73.6 ± 22.7 |
| Ethanol insoluble | 122.2 ± 8.7 | 116.0 ± 19.3 | 165.8 ± 3.4 | 106.2 ± 17.0 | 127.8 ± 1.8 | 138.8 ± 29.4 | 113.5 ± 16.7 | 118.2 ± 15.5 |
| Total uptake | 148.3 | 171.7 | 235.6 | 192.9 | 238.1 | 231.8 | 192.8 | 208.3 |

rates at 40‰. Rates at 10‰ approached the low levels observed for the animals at 40‰, while those at 20‰ were only slightly less than those of the scyphistomae at 30‰.

Mean dry weights (μg/polyp) are given in Table I. Absolute values (expressed as cpm/μg dry weight) for $C^{14}O_2$, labeled FAA, ethanol insoluble radioactivity and total radioactive glycine uptake are given in Table I. When corrected for machine efficiency, 100 cpm are equivalent to approximately 0.6 pico Moles of $C^{14}$ glycine. Values for the various fractions expressed as per cents of the total uptake at a given salinity and time since feeding are given in Figure 1.

*Recently fed (48 h) polyps*

Both the total glycine uptake and the size of the labeled FAA pools of recently fed scyphistomae increased (on absolute and relative bases) with salinity from 10 to 30‰ (Table I; Fig. 1); this relationship appears to be widespread among

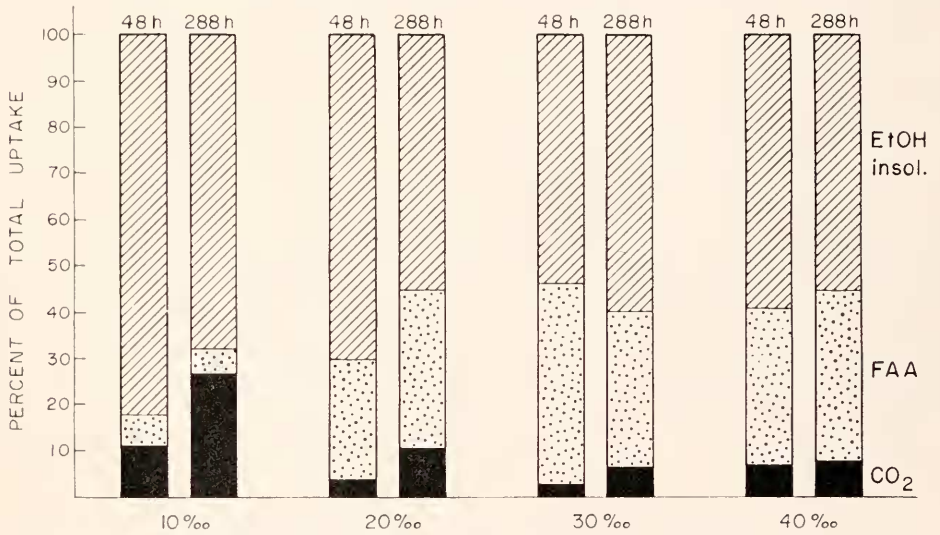


FIGURE 1. Distribution and utilization of $C^{14}$ glycine by *Aurelia* polyps, expressed as per cents of total uptake at various salinities and times since feeding.

euryhaline marine invertebrates. The sixfold increase in radioactivity present as FAA in Corpus Christi polyps at 20‰ when compared to animals at 10‰ closely parallels the change in glycine levels of the total FAA pools of Chesapeake Bay polyps 48 hours after feeding reported by Webb *et al.*, (1972). Similarly, the reduced size of the free glycine pool at 40‰ (Table I) is again paralleled by the decline of glycine in the pools of Chesapeake Bay polyps above 30‰; the decrease probably reflects, as do the depressed growth and reproductive rates of the Corpus Christi polyps, the effects of physiological stress at elevated salinities.

The relative amounts of label present as ethanol insoluble material showed an inverse relationship with salinity between 10 and 30‰ (Fig. 1). This phenomenon has been noted in a variety of marine and estuarine organisms (Stephens, 1964;

Stephens and Virkar, 1966; J. W. Anderson, personal communication), and probably serves to reduce the amount of osmotically active small molecules in the tissues at lower salinities.

The high levels of $C^{14}O_2$ production at 10 and 40‰ (Table I; Fig. 1) may again indicate stress at salinity extremes, although detailed metabolic studies would certainly be necessary to clarify this point; both groups of polyps exhibited depressed growth and reproductive rates. It may be significant with respect to this hypothesis that the lowest $C^{14}O_2$ production was observed at 30‰, the optimum salinity for growth and reproduction in the present study.

*Starved (288 h) polyps*

In comparisons with 48 h polyps, Student's $t$ tests revealed no significant differences ($P > 0.05$) in total $C^{14}$glycine uptake after starvation at any experimental salinity. The production of $C^{14}O_2$ by polyps 288 hours since feeding is from two to three times greater than that of the more recently fed animals at 10, 20, and 30‰ (Table I; Fig. 1). Production of $C^{14}O_2$ increased (on absolute and relative bases) in starved polyps at 10‰, while free $C^{14}$glycine remained at the same low level (Fig. 1). At 20‰, $C^{14}O_2$ produced by starved animals increased, while free labeled glycine again remained essentially the same as in the 48 h polyps. The increase in labeled $CO_2$ at 30‰ occurred with a concomitant decrease in the amount of free labeled glycine (Table I; Fig. 1). Total glycine uptake by starved polyps at 40‰ matched that of recently fed scyphistomae (Table I), and it is noteworthy that the relative levels of all fractions also remained constant (Fig. 1). The apparent lack of starvation effects at 40‰ may indicate a minimum critical level of glycine uptake and an optimum distribution of the amino acid among catabolic pathways, FAA pools and synthesis of larger molecules at this stressful salinity that remain constant even during the added stress of 288 hours of food deprivation.

## Discussion

At this point, explanations of the above observations must be largely speculative. Webb *et al.* (1971) have demonstrated that starved turbellarians decrease the amount of FAA released into the medium, although these authors present no data concerning the rate of uptake under similar conditions. Unfortunately, the total amino acid flux in scyphozoan polyps, either recently fed or starved, has not been determined, although the results of the present study indicate that the rate of glycine uptake is essentially unaffected by 288 hours of food deprivation. Thus, the often observed net outward flow of FAA may be reversed to some degree during starvation.

The low level of free $C^{14}$ glycine in 48 h and 288 h polyps at 10‰ is suggestive of an optimum and necessarily low concentration of an osmotically active amino acid at reduced salinity that remains constant even during starvation. The absolute values and percentages of free glycine in both 48 h and 288 h polyps at 20 and 40‰, and in 288 h polyps at 30‰, are very similar (Table I; Fig. 1;) this similarity over such a range of salinity perhaps indicates that the pools serve as

sources for a metabolic pathway or pathways, rather than having a strictly osmotic function. The larger free glycine pool in 48 h polyps at 30‰ may be correlated with the observed maximum growth and reproductive rates (and hence, presumed maximum metabolic efficiency) at this salinity; these polyps therefore accumulate large amounts of this amino acid into FAA pools, channeling much of the pool into catabolic pathways during food deprivation, a supposition that is supported by the work of Raum (1970). Further, the glycine that is taken up from solution during starvation at this salinity is broken down fairly rapidly, rather than remaining in the large pools observed in the recently fed animals.

The two-to-threefold increases in $C^{14}O_2$ production by starved polyps when compared to recently fed animals are probably explained by the larger percentage of labeled glycine relative to unlabeled glycine in the FAA pools of the starved animals. However, since the amount of incorporation of labeled glycine into ethanol insoluble material either decreases or remains essentially unchanged after starvation, the possibility of an enhanced rate of glycine oxidation by starved polyps exposed to dissolved glycine cannot be discounted. Whichever is the case, there is proportionately greater participation of exogenous glycine in the catabolic pathways of the starved scyphistomae. The estimation of the significance of this increase must await the determination of the total glycine flux in these animals. It must also be pointed out that the $C^{14}O_2$ produced is not necessarily respiratory in origin, for Greenberg (1961) has indicated that the catabolism of glycine via serine and pyruvic acid and subsequent entry into the tricarboxylic acid cycle is of little quantitative significance in vertebrate liver. A similar situation seems to exist in scyphozoan polyps; more than 99% of the radioactivity recovered from *Chrysaora quinquecirrha* polyps exposed to $C^{14}$ glycine was still present as glycine, while the radioactivity recovered from polyps exposed to labeled serine was determined to be 73.8% in serine and 20.7% in glycine (Dr. K. L. Webb, personal communication).

Previous investigations of a nutritive role of dissolved organic compounds have been performed using well fed animals having presumably high levels of metabolic substrates. The present investigation has demonstrated that the oxidation of glycine taken up from solution assumes increased importance when substrates derived from prey or other solid food may be limiting.

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## Summary

1. The size of the labeled free glycine pools in recently fed *Aurelia aurita* polyps exposed to dissolved $C^{14}$ glycine increased with salinity from 10 to 30‰; the decline at 40‰ is probably a reflection of stress on the animals.

2. The percentage of radioactivity present as ethanol insoluble material was inversely related to salinity between 10 and 30‰.

3. The rate of glycine uptake was unaltered after 288 hours of food deprivation at 10, 20, 30 and 40‰.

4. The oxidation of radioactive glycine taken up from solution, as measured by the collection of $C^{14}O_2$, increased two- to threefold in starved polyps at 10, 20 and 30‰.

5. It is suggested that experiments utilizing starved animals, in which substrates derived from solid food are low, are of importance in elucidating the role of dissolved organic compounds as supplemental energy sources for marine invertebrates.

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